Remarks

Claims 1-12 are pending in the present application. Claims 1-12 have been rejected. By the present amendment, claims 1-12 are canceled and replaced with new claims 13-49. The new claims have been introduced to advance prosecution of the present application, which had been translated from the German language. No new matter has been added.

In addition, the specification has been amended to conform to U.S. practice and to correct certain errors. No new matter has been added. A marked-up copy of the Substitute Specification showing the changes made is enclosed herewith.

Rejections Pursuant to 35 U.S.C. §§102 & 103

In the Office Action, claim 8 was rejected under 35 U.S.C. §102(b) as being anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over either Karvinen or Michalczyk et al. Claim 8 was also rejected under §102(e) as anticipated by or, in the alternative, under §103(a) as obvious over either Horne et al. or Murayama et al. Further, claims 9-12 were rejected under §103(a) as being unpatentable over Horne et al. or Murayama et al. or Karvinen or Michalczyk et al.

Claims 1-12 are cancelled herein, therefore mooting the instant rejections. Applicants respectfully request the rejections be withdrawn.

Claim Rejections Pursuant to 35 U.S.C. §112, Second Paragraph

Also in the Office Action, claims 1-12 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. In addition, claims 9-12 were rejected under 35 U.S.C. §101 for recitation of a use without setting forth any steps involved in the

process. Claims 4-12 were rejected under 35 U.S.C. §112, paragraph 5 in constituting multiple dependent claims which do not depend from the parent claims in the alternative only, and claims 5-12 were further rejected in constituting multiple dependent claims which depend from other multiple dependent claims.

Claims 1-12 are cancelled herein, mooting the instant rejections. Moreover, new claims 13-49 do not contain the language which the Examiner found objectionable and comply with the various statutes. Applicants respectfully request the rejections be withdrawn.

Applicants gratefully acknowledge the Examiner's indication of allowable subject matter and respectfully request that the new claims be allowed.

Conclusion

Applicants have filed a complete response to the outstanding Office Action and respectfully submit that, in view of the above amendments and remarks, the application is in condition for allowance. The Examiner is encouraged to contact the undersigned to resolve efficiently any formal matters or to discuss any aspects of the application or of this response. Otherwise, early notification of allowable subject matter is respectfully solicited.

Respectfully submitted,

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MODIFIED OXIDIC NANOPARTICLE WITH HYDROPHOBIC INCLUSIONS AND PROCESS FOR PRODUCING SAME

BACKGROUND OF THE INVENTION

[001] The present invention concerns processes for producing modified metal-oxidic nanoparticles with hydrophobic inclusions and, more particularly, metal oxide particles which contain halogen-containing target molecules, and the particles produced in this manner. The particles have application, for example, as a toner, sunscreen agent, insecticide or for labeling biomolecules.

[002] Latex particles are hydrophobic and are very suitable as a host for hydrophobic molecules and used as such (Kawaguchi, H., Prog. Polym. Sci. 25 (2000) 1171-1210). Although organic nanoparticles dispersed in water are being used increasingly in pharmaceuticals, cosmetics, plant protection and foods, solvent residues are for example still present which can have an adverse effect on the respective applications. These problems are at present being intensively researched (Horn, D., and Rieger, J., Angew. Chem. 113 (2001) 4460-4492).

[003] Since the metal oxide particles are synthesized by wet chemical methods in a water-ethanol mixture they naturally contain no interfering surfactants, stabilizers, etc. In addition, a multifunctional surface is present which can be modified depending on the requirements, for example, with carboxyl functionalities (as biolinkers) or fluoro-organyl groups (to influence the physicochemical surface properties). Metal oxide particles are by nature hydrophilic and are therefore unsuitable as a host for hydrophobic molecules.

[004] Nanoparticles based on silicate which are stained with hydrophilic dyes are known. They are used in the form of pigments, for example, as dyes for toners and inks, for plastic materials, and also as labeling and carrier materials in the medical engineering field.

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[005] On an industrial scale silicate particles are usually produced by flame hydrolysis (e.g., Aerosil®). Silicate particles obtained in this manner can be colored on their surface or in layers.

[006] U.S. Patent No. 5,102,763 describes the use of hydrophilic, colored SiO_2 particles for use as toners. The surface of these particles is covalently stained by reacting pre-activated silicate particles with various dyes.

[007] The production of colored particles by covalently binding a dye to the surface of particles is described in International Publication No. WO 93/10190.

[008] Silicate particles which are only colored on the surface have a tendency to lose color by bleeding. This results in a reduction in the color intensity and these particles are also often no longer uniformly colored. The use of these particles to produce conjugates that are suitable for diagnostic agents is not described.

[009] A process for producing colored particles with a silicate surface is described in U.S. Patent No. 5,209,998. The production process is based on the coating of colored pigments with a silicate shell. Hence, only the nucleus of these particles is colored. The use of the particles in electrostatic toners, plastic materials and inks is described as the application; a diagnostic application is not disclosed.

[0010] A process for producing monodisperse silicate particles, i.e., silicate particles of a uniform size, is the sol-gel process. It was first described by Stoeber et al., (Colloid J. Interface Sci. 26 (1968) 62-69). The production of so-called Stoeber particles and their properties were subsequently extensively examined by numerous groups. These studies encompassed the determination of the synthesis conditions required to obtain certain particle sizes (Van Helden, et al., Colloid J. Interface Sci. 81 (1981) 354-68; Giesche, H., J. European Ceramic Soc. 14 (1994) 189-204; Van Blaaderen, A., and Vrij, A., Adv. Chem. Ser. 234 (1994) 83-111) as well as investigations on particle growth and chemical composition (Byers, C.H., et al., Ind. Eng. Chem. Res. 26 (1987) 1916-1923;

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Matsoukas, T., and Goulari, E., Colloid J. Interface Sci. 124 (1988) 252-261; Harris, T., et al., J. Non-Cryst. Solids 121 (1990) 307-403; Matsoukas, T., and Gulari, E., Colloid J. Interface Sci. 132 (1989) 13-21; Badley, R.D., et al., Langmuir 6 (1990) 792-801).

[0011] Various methods have been described in the prior art for doping silicate particles from the sol-gel process with dyes.

[0012] Van Blaaderen, et al., Langmuir 8 (1992) 2921-2931 and Quellet, et al., Colloid J. Interf. Sci. 159 (1993) 150-7 produced Stoeber particles that were stained with fluorescein isothiocyanate or rhodamine isothiocyanate (Verhaegh and Van Blaaderen, A., Langmuir 10 (1994) 1427-1438). The dyes were previously reacted with 3-aminopropyltriethoxysilane (AMEO). In this case the dye was covalently attached to the surface or covalently incorporated into the particles in layers. The resulting inhomogeneous staining was of secondary importance in these investigations and the method usually resulted in relatively large particles in a size range of about 500 nm diameter. The particles obtained were used as model systems for basic research. The large particle size makes silicate particles produced in this manner less suitable for diagnostic applications.

[0013] Shibata, S., et al., J. Sol-Gel Sci. and Techn. 10 (1997) 263-268 physically doped Stoeber particles with various hydrophilic dyes such as rhodamine 6G, water-soluble porphyrins, Nile-blue, etc. Schwert, R., Dissertation Wuerzburg 2000 found that only cationic but not anionic or hydrophobic dyes can be incorporated physically (non-covalently) in the Stoeber process.

[0014] Matijevic, et al., Dyes and Pigments 17 (1991) 323-340 presented Stoeber particles whose surface was modified with 3-aminopropyltriethoxysilanes which were linked via the amino group with dyes in a complicated process. The surface of Stoeber particles was also modified in various other manners. These include reactions with 3-methacryloxypropyltrimethoxysilane (MEMO), octadecyltrimethoxysilane (ODS) and 3-aminopropyltriethoxysilane (AMEO) (Giesche, H., and Matijevic, E., Dyes and Pigments

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17 (1991) 323-340; Van Blaaderen, A., and Vrij, A., Golloid J. Interface Sci. 156 (1993) 1-18; Badley, R.D., et al., Langmuir 6 (1990) 792-801; Philipse, A.P., and Vrij, A., Colloid J. Interface Sci. 12 (1989) 121-136; Van Helden, A.K., and Vrij, A., Colloid J. Interface Sci. 81 (1981) 354-368).

[0015] Homogeneously colored silicate particles can be produced in the sol-gel process by covalent dye incorporation (European Patent No. 1 036 763 B1). However, the dyes have to be firstly silanized before they can be used in this process. A covalent incorporation is only possible in this manner.

[0016] Many important target molecules for incorporation into nanoparticles and in particular many dyes carry halogen groups as substituents. These dyes are not only hydrophobic but also oleophobic.

[0017] Fluorine-containing coatings based on SiO_2 are known (Lotus-Effect, Easy to clean surfaces, adjustment of refractive numbers – Kron J., et al., 2nd Woerlitzer Workshop: Functional layers – adhesive and antiadhesive surfaces ("Foerdergemeinschaft Duenne Schichten e.V."), Conference paper 2000). However, due to the rapid gelling during the particle production with fluoroalkyltrialkoxysilanes, no fluorine-containing silicate particles have yet been synthesized. But these would be desirable in order to also enclose hydrophobic and especially oleophobic molecules in SiO_2 particles.

SUMMARY OF THE INVENTION

[0018] It is against the above background that the present invention provides certain unobvious advantages and advancements over the prior art. In particular, the inventor has recognized a need for improvements in processes for producing modified oxidic nanoparticles with hydrophobic inclusion.

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[0019] Although the present invention is not limited to specific advantages or functionality, it is noted that the present invention provides processes for producing metal oxide particles in the sol-gel process in the presence of fluoroorganylalkoxysilane or arylalkoxysilane and to non-covalently incorporate hydrophobic and in particular oleophobic target molecules into these nanoparticles in this production process.

[0020] In accordance with one embodiment of the present invention, a sol-gel process for producing a metal oxide particle is provided which contains at least one target molecule containing halogen in which, starting from known metal oxide precursors, the precursor and the target molecule are used, characterized in that a polyhalogenated metal alkyl-alkoxy compound, in particular alkylalkoxysilane is additionally used in the sol-gel process. More particularly, in accordance with the instant embodiment, a sol-gel process for producing a metal oxide particle is provided comprising a) providing a mixture comprising a halogen-containing target molecule and a polyhalogenated metal alkylalkoxy compound; b) starting a sol-gel process with an initial amount of a metal oxide precursor; c) adding the mixture from a); and d) ending the sol-gel process.

[0021] These and other features and advantages of the present invention will be more fully understood from the following detailed description of the invention taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The following detailed description of the embodiments of the present invention can be best understood when read in conjunction with the following drawings, where like structure is indicated with like reference numerals and in which:

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[0023] Fig. 1 is a schematic representation of homogeneous (type 1), heterogeneous (core-shell (type2)), and currant cake (type 3) particles having an inorganic-oxidic matrix;

[0024] Fig. 2 shows the structural formula of Tris-[4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione]-Eu(III) complex (Eu(NTA)3 complex);

[0025] Fig. 3 is a plot of the UV-Vis spectrum of the Eu(NTA)3 complex in CH_2Cl_2 / EtOH (1:1);

[0026] Fig. 4 is a plot of the fluorescence spectrum of the EU(NTA)3 complex in CH_2Cl_2 / EtOH (1:1);

[0027] Fig. 5 is a plot of the measurement of a solid specimen of silicate particles containing the Eu(NTA)3 complex which were fixed on a microscope slide;

[0028] Fig. 6 is a plot of the IR spectrum of the silicate particles doped with the Eu(NTA)3 complex on a pressed piece of KBr;

[0029] Fig. 7 is a plot of the Raman spectrum of a solid specimen of silicate particles doped with an Eu(NTA)3 complex;

[0030] Figs. 8a and 8b show a pair of TEM pictures of 130-158 nm silicate particles doped with 4.9 µmol Eu(NTA)3 complex per g SiO_2 at 6300-fold and 63000-fold enlargement, respectively;

[0031] Fig. 9 is an illustration of the VACP/MAS 13C solid NMR spectrum of the Eu(NTA)3 complex; and

[0032] Fig. 10 is an illustration of the MAS ^{29}Si solid NMR of silicate particles doped with the Eu(NTA)3 complex.

DETAILED DESCRIPTION OF THE INVENTION

[0033] A sol-gel process is understood as any process which can be used in analogy to the process described by Stoeber et al. (1968), *supra* to produce colloidal nanoparticles. The products of this process are referred to as Stoeber particles or nanoparticles.

[0034] The invention concerns the non-covalent incorporation of halogen-containing target molecules into metal oxide particles. The target molecules in the sense of this invention consist of 5 – 65 percent by weight (= weight %) halogen and typically have a molecular weight of between 250 and 5000 Dalton. Target molecules are in particular halogen-containing dyes and halogen-containing insecticides.

[0035] The halogen-containing target molecule is not silanized. Hence their incorporation into the Stoeber particles is non-covalent.

[0036] The process according to the present invention is especially characterized in that for the first time it is possible to produce Stoeber particles in the presence of a polyhalogenated metal alkylalkoxy compound. The process can be carried out in the presence or absence of a target molecule. A polyhalogenated metal alkylalkoxy compound contains a linear or branched alkyl residue with 2 to 20 carbon atoms which carries at least two halogen groups. The polyhalogenated alkyl residue typically contains less than 30 halogen groups. More typical polyhalogenated metal alkylalkoxy compounds contain alkyl residues with 3 to 20 carbon atoms and 2 to 15 halogen groups. Metal alkylalkoxy compounds based on silicon, titanium or zirconium and, in particular, alkylalkoxysilanes are more typical.

[0037] Metal alkoxides or metal halogenides are usually used as metal oxide precursors. Typical metal alkoxides are silicon metal oxides, in particular, tetraethoxysilane (TEOS) and tetramethoxysilane (TMES).

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[0038] In the original Stoeber process using SiO_2 as the metal oxide, the SiO_2 particles are produced by hydrolysis and condensation of a silicon alkoxide which is usually tetraethoxysilane (TEOS). The reaction takes place in a mixture of water, ammonia and a lower alcohol, usually ethanol. The main reactions in the formation of the SiO_2 particles can be described as follows:

[0039] 1) Hydrolysis

$$\equiv Si—OR + H_2O \rightarrow \equiv Si—OH + ROH$$

[0040] 2) Condensation I

$$\equiv Si—OR + \equiv Si—OH \rightarrow \equiv Si—O—Si\equiv + ROH$$

[0041] 3) Condensation II

$$\equiv Si—OH + \equiv Si—OH \rightarrow \equiv Si—O—Si\equiv + H_2O$$

[0042] 4) Total reaction

$$Si(OR)_4 + 2H_2O \rightarrow SiO_2 + 4\ ROH$$

[0043] In the synthesis alcohol, water and ammonia are added first and subsequently TEOS is added. Depending on the synthesis conditions, the solution becomes opalescent after a few seconds to minutes. This induction period increases with decreasing particle size and temperature. The size of the particles obtained has a standard deviation of 2-8 %. During the reaction the alcohol serves as a cosolvent for the water-insoluble TEOS. The ammonia catalyses the hydrolysis as well as the condensation reaction. The base deprotonates the surface silanol groups of the formed particles. The resulting negative charges stabilize the colloidal system as a result of electrostatic repulsion. Hence, the suspensions remain stable for several months to years. At the same time the silanol groups that are present enable a functionalization of the particle surface (various examples thereof have already been described in the

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literature, e.g., AMEO, ETEO, MEMO, MPTMO, GLYMO, GF20). Their dispersibility in various solvents can be varied by suitable surface modification.

[0044] The particle size can be controlled by the ammonia and water concentration, the reaction temperature, and the solvent. The following trends are seen:

[0045] 1) Increasing the ammonia as well as the water concentration accelerates the reaction and increases the particle size.

[0046] 2) The particle size increases and the monodispersity decreases with increasing chain length and branching of the alcohol.

[0047] 3) The particle size decreases with an increasing length of the alkoxide residues of the silane. The ionic strength of the reaction solution (salt effect) can also influence the particle size due to compression of the electrostatic double layer.

[0048] 4) If the TEOS concentration exceeds 0.2 mol/l, the particles become more polydisperse and less spherical.

[0049] The previously discussed influences on particle formation in the original Stoeber process apply analogously to the process according to the present invention in which a polyhalogenated metal alkylalkoxy compound is additionally used in order to, for example, incorporate a halogen-containing target molecule in the Stoeber particles obtained by this process.

[0050] The process according to the present invention can, for example, be carried out by simultaneously reacting the metal oxide precursor, halogen-containing dye and polyhalogenated metal alkylalkoxy compound components under suitable reaction conditions known to a person skilled in the art.

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[0051] The halogen-containing target molecule and the polyhalogenated metal alkylalkoxy compound are typically dissolved in advance in a suitable solvent, mixed and added together.

[0052] A sol-gel process comprising the following steps is more typical for producing a metal oxide particle containing at least one halogen-containing target molecule: a) production of a mixture containing the target molecule and a polyhalogenated metal alkylalkoxy compound, b) starting the sol-gel process with a metal oxide precursor, c) adding the solution from a), d) optionally further addition of the metal oxide precursor and e) ending the sol-gel process.

[0053] The quantity ratios of the metal oxide precursor that are used in the above steps b) and d) can vary over a wide range. Typically between about 90 to about 10 % of the total amount of metal oxide precursor used in the process is used in step b) and correspondingly the remaining about 10 to about 90 % is used in step d). The partial amount used in step b) is more typically about 75 to about 25 % and in step d) about 25 to about 75 %.

[0054] Also, the time period for starting the sol-gel process in step b) is variable. It is typically less than about 1 h, more typically between about 1 and about 20 min and even more typically between about 2 and about 10 min.

[0055] It has proven to be particularly suitable to coordinate the molar ratios of metal oxide precursor and polyhalogenated metal alkylalkoxy compound. Typically about 0.04 to about 0.4 mol % polyhalogenated metal alkylalkoxy compound, more typically about 0.1 to about 0.3 mol % based on the metal oxide precursor are used.

[0056] The halogen-containing target molecules typically contain between about 5 and about 65 % weight, more typically between about 15 and about 50 weight % halogen and the molecular weight is typically between about 250 and about 5000 Dalton, more

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typically between about 300 and about 4000 Dalton and even more typically between about 400 and about 3000 Dalton.

[0057] Typical halogens in the halogen-containing target molecules are fluorine and chlorine.

[0058] The amount of added target molecule can vary according to needs. Of course it is also possible to prepare particles which contain no target molecules or only minimal amounts thereof. Typically between about 0.1 and about 10 % by weight target molecule and more typically between about 0.2 and about 5 weight % based on the metal oxide precursor is used.

[0059] Oxides of the elements from groups III, IV and IVb of the periodic system come into special consideration as metal oxides or as components of mixed oxides. The metal oxide precursor is typically selected such that in addition to the inclusions of the target molecule and the covalently incorporated polyhalogenated metal alkyl, the Stoeber particles can be selected from B_2O_3, Al_2O_3, SiO_2, SnO_2, ZrO_2, TiO_2, or

[0059] combinations thereof. As such, particles based on mixed oxides can be used in an analogous manner in the sol-gel process of the present invention.

[0060] Metal oxide precursors based on boron, silicon or zirconium are typically used, silicon precursors being more typical.

[0061] The present invention also concerns the particles that can be obtained by the process according to the invention. These are in particular particles which were obtained by hydrolysis and condensation of sol-gel precursors of elements of groups III, IV, IVb, typically Si (Ti, Zr, Al) in combination with hydrophobic sol-gel precursors such as perfluorinated alkyltri-alkoxysilanes (e.g., 3,3,3-trifluoropropyltrimethoxysilane) or bis(trialkoxysilyl-alkyl)benzenes (e.g., bis(trimethoxysilylethyl)benzene). These particles typically contain the above-mentioned target molecules.

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[0062] Due to the hydrophobic (fluorinated) environment that is present in the case of the metal oxide particles produced according to the present invention, fluorophores for example do not exhibit the otherwise common adverse effects of water, i.e., quenching due to water does not occur.

[0063] The modification of the particle interior according to the invention enables other hydrophobic (e.g., LC Red 640) as well as oleophobic molecule/complexes to be incorporated into the originally highly polar oxidic matrix in addition to lanthanoid complexes. The particle surface can be functionalized, for example, with carboxyl, amino, mercapto, epoxy and aldehyde groups. This can be accomplished among others by silanization. The particle size can be adjusted from the nano- to micrometer range with a narrow size distribution.

[0064] The particle type (cf., Fig. 1) is not decisive. The particles are typically composed of an inorganic-oxidic core. This core can have a homogeneous (type 1) or heterogeneous (core-shell type (2) or currant cake model (3)) composition.

[0065] The Stoeber particles according to the present invention loaded with a halogen-containing target molecule can be very advantageously used in various technical fields. For example, they are especially suitable as labels for biomolecules and hence for applications of the labeled biomolecules in immunological and other detection methods, as toners in the printing industry, as sunscreen agents and as insecticides. It is also possible to incorporate them into any polymer matrix (e.g., Ormocer®). The applications as labels for biomolecules or as an insecticide are typical.

[0066] The metal oxide particles according to the present invention can be subsequently modified. Thus, the particles can, for example, be coated with one or more additional, typically colorless, layers in order to chemically protect the particles. The purpose of this coating is to obtain a metal oxide surface, e.g., a silicate-like surface that is as uniform as possible from which color molecules no longer protrude. This facilitates additional coupling with functional groups and biomolecules and reduces the risk of secondary

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reactions with dye molecules on the surface. Typically, an additional uncolored silicate layer is applied at a thickness of about 1 to about 30 nm, typically about 2 to about 20 nm to the homogeneously colored silicate particles.

[0067] The metal oxide particles according to the present invention can either be provided directly with functional groups or they can be provided on the surface of the additional coating layer in order to couple additional molecules to the particle which according to the invention are typically biomolecules.

[0068] The functional groups can in turn be attached to the particles via spacer or linker molecules. Typically, the functional group to be introduced is anchored in the network of the metal oxide particle in order to ensure a stable linkage.

[0069] Typical modification groups are functional groups such as carboxyl groups, amino groups, epoxy groups, hydroxyl groups or thiol groups. A person skilled in the art knows how to introduce such groups.

[0070] It is typical to introduce carboxyl groups which is typically carried out by reacting the colored metal oxide particles with a dye acid anhydride which contains the silanol group for anchoring in the particle. In order to activate the functional groups they can for example be converted into active esters with N-hydroxy-succinimide before reaction with the biomolecules to be coupled. All these steps are familiar to a person skilled in the art.

[0071] The conjugates according to the present invention are composed of metal oxide particles loaded with a halogen-containing dye and biomolecules. The biomolecules are typically coupled via the functional groups that are introduced on the surface. In general, the biomolecules are linked to the surface of the particle via free amino or carboxyl groups or thiol groups such that the covalent linkage is typically via amide or thioether bonds.

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[0072] Biomolecules in the sense of the present invention are understood as all molecules that can be used to determine an analyte in a sample, in particular, for an immunological determination of an analyte. The term biomolecule, for example, includes proteins, glycoproteins, peptides, nucleic acids, peptidic nucleic acids, saccharides, hormones, haptens, vitamins, naturally occurring or artificially produced binding partners and antigens. Antibodies and fragments thereof are typically used as biomolecules in the conjugate according to the present invention. Antibodies are understood to include monoclonal as well as polyclonal antibodies and chimeric antibodies and fragments thereof such as, for example, Fab, Fc, Fab', $F(ab')_2$, Fv, and scFv. Coupling to the biomolecules streptavidin or avidin or biotin is also one of the typical embodiments of the invention.

[0073] Conjugates of the inventive metal oxide particles and biomolecules are a further subject matter of the invention. These inventive conjugates are typically used in a method for detecting an analyte in a sample by contacting the sample with one or more analyte-specific binding partners.

[0074] The method for detecting an analyte is typically carried out as an immunoassay. This means that at least one of the analyte-specific binding partners is an immunological binding partner. In this method the sample which is presumed to contain the analyte is incubated with an immunologically specific binding partner. In the case of an antigen test, for example, for tumour markers such as PSA, an antibody or a fragment thereof which specifically binds to the analyte, i.e., the tumour antigen PSA, is the immunologically specific binding partner. In methods for detecting antibodies to a certain antigen (e.g., anti-HCV antibodies) the corresponding antigen can for example be used as the immunologically specific binding partner. The specific binding is detected by means of the inventive conjugate whose incorporated dye serves as a label. The biomolecules immobilized on the metal oxide particles act as specific binding partners for the analyte or as specific binding partners for a substance which in turn is specifically bound to the analyte.

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[0075] For example, in a diagnostic test procedure, streptavidin or avidin can be conjugated as the biomolecule to the metal oxide particle. The conjugate then binds to the biotin group of a molecule (for example, a peptide antigen or a nucleic acid sequence) that is itself biotinylated.

[0076] Immunoassay procedures and nucleic acid test procedures are familiar to a person skilled in the art.

[0077] The conjugates according to the invention are typically used in a test based on a test strip. The following describes, as an example, how a test strip is constructed and how such a test procedure is carried out.

[0078] Test strips are usually composed of a carrier material on which an application fleece, a membrane, and a suction fleece are mounted. The conjugate according to the present invention whose biomolecules are specific for the analyte and optionally other specific binding partners for the analyte are applied and dried upstream of the chromatography direction, i.e., above the starting point for the sample liquid. The specific binding partners and the inventive conjugate do not begin to migrate chromatographically until contact with a liquid, i.e., with the sample. Various proteins are also applied to the membrane in the direction of chromatography in the form of two successive strips or lines.

[0079] An immobilized binding partner that is specific for the analyte is located on the first line (result line). A molecule such as streptavidin can also be bound to the first line to which biotinylated, analyte-specific binding partners can then bind. In this case, the biotinylated, analyte-specific binding partners as well as the conjugate are applied above the starting point of the test strip and chromatographed together with the sample. A binding partner which specifically binds the biomolecules of the inventive conjugate is applied to the second line in the direction of chromatography (control line).

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[0080] As the sample liquid migrates from the starting point of the test strip through the strip, the conjugate according to the present invention and optionally the analyte-specific binding partner also begin to migrate towards the liquid front. In this process the analyte from the sample specifically binds to the binding partners immobilized on the first line. The inventive conjugate also binds to the analyte to form a sandwich that can be detected by means of the color of the metal oxide particles. The liquid in the test strip runs further up to the end of the test strip. In this process the inventive conjugate that is not consumed by analyte binding is captured on the second line by the binding partner that specifically binds the biomolecules of the conjugate. One can see on the basis of the coloration of the control line that the chromatography in the test strip has basically worked and/or is completed.

[0081] Another subject matter of the invention is a diagnostic test strip which, in addition to the conjugate according to the invention, contains all other components necessary to carry out the chromatographic test.

[0082] According to the invention the conjugates can also be used in nucleic acid hybridization assays. In this case a nucleic acid probe which specifically hybridizes with a nucleic acid sequence to be detected is coupled as a biomolecule with the metal oxide particles that are colored according to the invention. The nucleic acid sequence from the sample or from a mixture that is for example obtained by PCR amplification can be specifically detected by means of the dye contained in the metal oxide particles.

[0083] According to the invention the conjugates comprising the metal oxide particles according to the invention and a biomolecule can also be used in array or chip systems. Such systems are miniaturized test designs. Spatially separated reagent spots are applied with a very small spacing which is in the micrometer range to the surface of suitable solid phases such as plastics, glass, metals or metal oxides. These reagent spots contain the specific binding particles required to carry out the respective detection method. Such detection methods enable numerous different analytical parameters to be detected simultaneously and rapidly in a very small space using little material and

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sample. The conjugates according to the invention comprising metal oxide particles colored with halogen-containing dyes and biomolecules can also be used as detection reagents in these array or chip systems. Suitable dyes for coloring the metal oxide particles are typically fluorescent dyes and especially those that enable a time-resolved measurement of fluorescence. In particular the conjugates according to the invention enable differently colored and/or different conjugates loaded with different biomolecules to be used in order to simultaneously detect different analytes by means of the different (fluorescent) dyes. Such array systems have proven to be particularly advantageous for nucleic acid hybridization assays.

[0084] The simultaneous detection of a plurality of different analytes (for example HIV- and HCV-specific nucleic acids in a sample or HIV- and HCV-specific antibodies in a sample) by means of the conjugates according to the invention which are each differently colored and/or loaded with different biomolecules is not limited to an application in array systems but is particularly appropriate therefore.

[0085] All body fluids can be used as the sample material for all diagnostic test methods. Whole blood, serum, plasma, urine, sweat or saliva are typically used.

[0086] Another subject matter of the invention is the use of the conjugates of metal oxide particles according to the invention and biomolecules in a diagnostic, typically immunological method to detect an analyte in a sample.

[0087] A diagnostic reagent which contains conjugates according to the invention is also a subject matter of the invention. The reagent can additionally contain the buffer additives, salts or detergents known to a person skilled in the art.

[0088] A test kit which contains the conjugates according to the invention and other common reagents known to a person skilled in the art for carrying out a test is also one of the typical embodiments of the present invention.

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[0089] Many important insecticides have a high halogen content. These insecticides are typical target molecules for the process according to the invention for non-covalent incorporation into metal oxide particles and in particular into silicate particles.

[0090] The digestive tract of insects and especially of insect larvae differs fundamentally from that of mammals. Whereas there is a strongly acidic pH the stomach of mammals, food is digested in the digestive tract of insect larvae in a strongly alkaline pH range.

[0091] Metal oxide particles especially those based on silicate or mixed oxide particles containing more than 20 % silicate have the special property that they swell under alkaline pH conditions such as those that are for example present in the digestive tract of insects and release non-covalently incorporated components, in particular polyhalogenated insecticides. Since the insecticide target molecules are not covalently bound in the particles according to the invention, they are released and are effective in the insect intestine i.e. precisely at the intended site of action.

[0092] The inclusion of insecticide agents in metal oxide particles by the sol-gel process of the present invention additionally has the effect that the agents are for example protected from water. The insecticidal effect only occurs after intake of food by the insect. Sol-gel particles according to the invention containing insecticides with incorporated insecticidal agents are less poisonous and/or more environmentally friendly than the free agents.

[0093] Halogen-containing dyes having spectral properties that are important for the printing industry can be incorporated into sol-gel particles in the process according to the invention. Such particles are used especially as an admixture in so-called toners.

[0094] The halogen-containing substances which can be incorporated according to the invention into metal oxide particles include many substances which absorb and suppress damaging UV light or release it again as longer wavelength less damaging

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light. Metal oxide particles according to the invention which contain such substances are typically used in the cosmetic industry especially as sunscreen agents.

[0095] In order that the invention may be more readily understood, reference is made to the following examples, which are intended to illustrate the invention, but not limit the scope thereof.

[0096] **Fig. 1**: Schematic representation of homogeneous (type 1), heterogeneous (core-shell (type2)), and currant cake (type 3) particles having an inorganic-oxidic matrix.

[0097] **Fig. 2**: Structural formula of Tris-[4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione]-Eu(III) complex (Eu(NTA)3 complex).

[0098] **Fig. 3**: UV-Vis spectrum of the Eu(NTA)3 complex in CH_2Cl_2 / EtOH (1:1) $\lambda_{abs.}$=333 nm.

[0099] **Fig. 4**: fluorescence spectrum of the EU(NTA)3 complex in CH_2Cl_2 / EtOH (1:1) $\lambda_{exc.}$= 333 nm.

[00100] Assignment of the fluorescence bands to the spectral transitions

Emission bands assignment*

- λ_{em} = 578 nm $^5D_0 \rightarrow {}^7F_0$
- λ_{em} = 590 nm $^5D_0 \rightarrow {}^7F_1$
- λ_{em} = 612 nm $^5D_0 \rightarrow {}^7F_2$
- λ_{em} = 651 nm $^5D_0 \rightarrow {}^7F_3$
- λ_{em} = 699 nm $^5D_0 \rightarrow {}^7F_4$

*Lit.: R. Reisfeld et al., J. of Alloys and Compounds 300-301 (2000), 147-151.

[00101] **Fig. 5**: Measurement of a solid specimen of silicate particles containing the Eu(NTA)3 complex which were fixed on a microscope slide.

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light source = xenon lamp

$\lambda_{exc.}$ = 333 nm

$\lambda_{em.}$ = 613 nm

[00102] **Fig. 6**: IR spectrum of the silicate particles doped with the Eu(NTA)3 complex on a pressed piece of KBr

Assignment of the bands

ν(O-H) = 3430 cm^{-1}

$\delta(H_2O)$ = 1640 cm^{-1}

ν(Si-O-Si) = 1100 cm^{-1} (as)

ν(Si-O-Si) = 800 cm^{-1} (sym)

δ(Si-O-Si) = 471 cm^{-1} (?)

Assignment according to: Fendler, J.H., Nanoparticles and Nanostructured Films, Wiley-VCH 1998, 180-183.

[00103] **Fig. 7**: Raman spectrum of a solid specimen of silicate particles doped with an Eu(NTA)3 complex

Assignment of the bands:

ν(C-H, aliph.) = 2943, 2875 cm^{-1} (as)

$\delta(CH_3, CH_2)$ = 1452 cm^{-1}

ν(Si-O-Si) = 1068 cm^{-1} (as)

ν(Si-O-Si) = 839, 793 cm^{-1} (sym)

δ(Si-O-Si) = 482 cm^{-1}.

Assignment according to: J.H. Fendler, *supra*.

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[00104] **Fig. 8:** TEM pictures of 130-158 nm silicate particles doped with 4.9 µmol Eu(NTA)3 complex per g SiO_2 at 6300-fold (Fig. 8 left) and 63000-fold enlargement (Fig. 8 right).

[00105] **Fig. 9:** VACP/MAS 13C solid NMR spectrum of the Eu(NTA)3 complex

Interpretation:

129.4 / 126.7 ppm; arom. C-H

61.2 ppm; CH_2-OH

27.6 ppm; CH_2-CH_2-CF_3

17.4 ppm; CH_3-CH_2-OH

4.5 ppm; Si-CH_2-CH_2-CF_3

[00106] **Fig. 10:** MAS ^{29}Si solid NMR of silicate particles doped with the Eu(NTA)3 complex.

Integration of the signals yielded the following distribution:

110.7 ppm; Q4-groups, 70.54 %

101.1 ppm: Q3-groups, 27.24 %

91.0 ppm: Q2-groups, 2.21 %

[00107] **Abbreviations used**

AMEO	3-aminopropyltriethoxysilane
<Dig>	anti-digoxigenin
ETEO	ethyltriethoxysilane
GF20	2(3-triethoxysilylpropyl)-succinic anhydride
GLYMO	glycidoxypropyltrimethoxysilane
Ig	immunoglobulin
LCR	LightCycler Red
MAB	monoclonal antibody
MEMO	methacryloxypropyltrimethoxysilane

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MES 2(N-morpholino)ethanesulfonic acid
MPTMO 3-mercaptopropyltrimethoxysilane
BPLA bovine plasma albumin
SA streptavidin
Si-NP silicate nanoparticle
TEOS tetraethoxysilane
TMES tetramethoxysilane

Example 1:

General protocol for preparing lanthanide (III)-tris-4,4,4-trifluoro-(1-naphthoyl)-1,3-butanedione complexes

[00108] 800 mg (3 mmol) 4,4,4-trifluoro-1-(2-naphthoyl)-1,3-butanedione was dissolved in 15 ml ethanol. Subsequently 3 ml of a 1 M NaOH solution was added to this solution. 1 mmol lanthanum (III) chloride or lanthanum (III) nitrate was dissolved in 5 ml water in a dropping funnel and then slowly added dropwise to the reaction solution. Afterwards a further 100 ml water was added to the reaction mixture and it was stirred for 1 hour at 65°C. The product was filtered off as a pale yellow solid and washed three times with 5 ml water and ethanol each time. It was finally dried for 3 hours at 120°C in a drying cabinet.

[00109] This general procedure was used to prepare terbium (III), gadolinium (III), dysprosium (III), and erbium (III) complexes.

Example 2:

Review of physical dye incorporation into fluorine-free ("normal") silicate nanoparticles (Si-NP) and organofluorine-modified (fluorinated) Si-NP

2.1 Preparation of normal silicate particles colored with LightCycler Red 640™ (reference particles)

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[00110] 41 mg ($4.29*10^{-5}$ mol) LightCycler Red 640™ LCR 640 was dissolved in 330 ml 99 % ethanol. 168 ml demineralized water and 11 ml of a 14 molar ammonium hydroxide solution were added to this solution. The solution was heated to 35°C. After a thermal equilibrium was established, 24 ml (107 mmol) tetraethoxysilane (TEOS) was added while stirring vigorously. The reaction was fully completed after 24 h. A colored dispersion having a solids content of about 2 % by weight was obtained. The particles have a size of about 135 nm diameter. These particles were purified of non-incorporated dye by centrifuging and redispersing three times in fresh ethanol.

2.2 Preparation of fluorinated silicate particles colored with LightCycler Red 640™

a) Protocol for particles containing 0.3 % fluoroalkylsilane

[00111] 23.8 µmol LightCycler Red 640™, then 6 ml TEOS and 30 µl (155 µmol) 3,3,3-trifluoropropyltrimethoxysilane (ratio of LCR 640™ : fluoroalkylsilane = 1:7) were added to a solution comprising 165 ml EtOH, 84 ml H_2O and 5.5 ml NH_4OH heated to 35°C. After stirring for 5 minutes the remaining 6 ml TEOS was added to the reaction mixture. The reaction was terminated after 8 h and the particles were separated by centrifugation. The particles were redispersed in H_2O and purified by centrifuging and redispersing several times.

b) Protocol for particles containing 0.2 % fluoroalkylsilane

[00112] 23.3 µmol LightCycler Red 640™, then 5 ml TEOS and 20 µl (119 µmol) 3,3,3-trifluoropropyltrimethoxysilane (ratio of LCR 640™ : fluoroalkylsilane = 1:5) were added to a solution comprising 31 ml EtOH, 20 ml H_2O and 7 ml NH_4OH heated to 30°C. After stirring for 5 minutes the remaining 5 ml TEOS was added to the reaction mixture. The reaction was terminated after 8 h and the particles were separated by centrifugation. The particles were redispersed in H_2O and purified by centrifuging and redispersing several times.

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2.3 Preparation of silicate particles doped with Eu(III)-tris-4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione

[00113] The Eu(III)-tris-4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione complex was prepared according to the instructions of Charles, R.G., and Roedel, E.P., J. Inorg. Nucl. Chem. 29 (1967) 715-723.

a) Simultaneous addition of alkoxide and a mixture of polyhalogenated alkylalkoxysilane and halogen-containing target molecule

[00114] 20 ml TEOS and a mixture of 20 mg tris-[4,4,4-trifluoro-1-(2-naphthyl)-1,3-butane-dione]-Eu(III), 1 ml dichloromethane and 0.25 ml 3,3,3-trifluoropropyltrimethoxy-silane were added to a solution comprising 61 ml water, 40 ml ethanol and 14 ml ammonium hydroxide solution heated to 30°C. The reaction mixture was stirred for 4 hours at 30°C and for a further 10 h at room temperature. The particles were purified by centrifugation and firstly redispersed in ethanol and then in water in a subsequent washing step.

[00115] Incorporation rate (complex): 4.9 µmol/g SiO_2

[00116] Calculated Eu content: 0.07 %

[00117] Eu content found by X-ray fluorescence analysis (RFA): 0.05 %

[00118] Particle size from TEM: 130-158 nm

b) Successive addition of alkoxide and a mixture of polyhalogenated alkylalkoxy-silane and halogen-containing target molecule

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[00119] 61 ml water and 40 ml ethanol were heated to 30°C in a 250 ml round bottomed flask. Then 14 ml ammonia solution and 10 ml TEOS were added. In parallel 19.4 mg (20.4 µmol) tris-[4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione]-Eu(III) was dissolved in 1 ml dichloromethane, and 250 µl trifluoropropyltrimethoxysilane was added to the solution in an ultrasonic bath. After 5 minutes the solution was added dropwise to the preparation and stirred for a further 5 minutes. Subsequently another 10 ml TEOS was added and the reaction mixture was stirred for 4 hours at 30°C and for a further 10 hours at room temperature. It was purified in several washing cycles using ethanol and water.

2.4 Preparation of the erbium (III) tris-(2,2'-bipyridyl)trichloride complex

[00120] 1.1 g (7 mmol) 2,2' bipyridine and 250 mg (0.7 mmol) erbium (III) nitrate (as an undefined hydrate complex) were added to 60 ml methanol. The reaction mixture was heated for 2 h to 60°C while stirring vigorously. The complex precipitated as a yellow powder on cooling.

[00121] Incorporation into silicate particles was carried out as described in Example 2.3a.

2.5 Preparation of the terbium (III) tris-(2,2'-bipyridyl)trichloride complex

[00122] 1.47 g (9.43 mmol) 2,2' bipyridine and 250 mg ($9.43 * 10^{-4}$ mol) terbium (III) chloride hexahydrate complex were added to 60 ml methanol. The reaction mixture was heated for 2 h to 60°C while stirring vigorously. The complex precipitated as a yellow powder on cooling.

[00123] Incorporation into silicate particles was carried out as described in Example 2.3a.

2.6 Preparation of the terbium (III) tris-(1,10-phenanthroline)trichloride complex

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[00124] 482 mg (2.67 mmol) 1,10-phenanthroline and 250 mg (0.94 mmol) terbium (III) chloride hexahydrate were added to 20 ml methanol. The solution was stirred for 2 h at 60°C and subsequently slowly cooled to room temperature (overnight). The yellow solution obtained in this manner was overlayered with n-pentane. The complex precipitates as a powder.

[00125] Incorporation into silicate particles was carried out as described in Example 2.3a.

2.7 Summary of the incorporation behaviour of various dyes into unmodified and halogen-modified silicate particles

	Incorporation into	
Incorporation of	normal Si-NP	fluorinated Si-NP
fluorine-free dyes	⊖ Tb(III)-bipy ⊖ Er(III)-bipy ⊖ Tb(III)-phen	⊖ Tb(III)-bipy ⊖ Tb(III)-phen
Fluorinated or halogenated dyes	⊖ $Eu(NTA)_3$ ⊖ LCR 640	⊕ $Eu(NTA)_3$ ⊕ $Er(NTA)_3$ ⊕ LCR 640

Legend:

⊖ incorporation negative,

⊕ incorporation positive

bipy = α,α'-bispyridine

phen = 1,10-phenanthroline

Example 3:

Surface modification with GF20 (protocol for introducing carboxyl groups)

[00126] The dispersion obtained in Examples 2.1 and 2.2 should not exceed a pH of 9.0. If necessary additional washing cycles have to be carried out (centrifugation /

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redispersion). 210 µl ($75.4*10^{-5}$ mol) 2-(3-triethoxysilylpropyl)-succinic anhydride (GF20) was added to the resulting ethanolic dispersion in a volume of 250 ml while stirring vigorously. The reaction solution was stirred for 15 h at 40°C. The particles were purified by centrifugation and redispersion in water. This purification step was repeated a further two times. An aqueous dispersion of surface-modified particles is obtained with a coverage density of ca. 2 CO_2H groups / nm^2 particle surface.

Example 4;

Preparation of conjugates of silicate particles colored with LightCycler Red 640 and anti-digoxigenin antibodies (<Dig> conjugates)

[00127] 10 mg silicate particles (0.5 ml 2 % suspension) was centrifuged for 30 min at 15000 rpm. The supernatant was removed and the pellet was resuspended in 1 ml 2 mM MES buffer pH 6.5. This washing process was repeated once more. Subsequently 100 µl 100 mM MES buffer pH 6.5, 100 µl 2 % (w/v) sulfo-N-hydroxy-succinimide (S-NHS; Pierce No. 24510) in MES buffer pH 6.5 and 100 µl 0.2 % (w/v) 1-ethyl-3-(3-diaminopropyl)-carbodiimide hydrochloride (EDC; Pierce No. 22980ZZ) in MES buffer pH 6.5 was added. After 20 min incubation period on a roller incubator, it was centrifuged for 30 min at 15000 rpm and the supernatant was taken off. The pellet was redispersed in 867 µl 2 mM MES buffer pH 6.5, and 133 µl MAB<Dig>M-IgG solution (monoclonal anti-digoxigenin IgG antibody from the mouse; concentration = 15 mg/ml) was added. Afterwards it was incubated for 2 h at room temperature (RT). Subsequently 1 ml of a 2 % solution of bovine plasma albumin (RPLA) in 5 mM potassium phosphate buffer pH 7.4 was added and it was incubated for a further 60 min at RT. The particle conjugates were centrifuged, the supernatant was removed and the pellet was resuspended in 1 ml 5 mM buffer. The potassium phosphate washing process was repeated twice and the particles were resuspended in 0.5 ml 2 % RPLA in 5 mM Hepes buffer pH 7.4 after the last centrifugation step.

Example 5;

Use of <Dig>silicate particle conjugates in a strip test

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[00128] The test strips required to carry out the experiments consist of a plastic foil onto which an application fleece, a membrane and a suction fleece are glued. Two proteins, streptavidin and anti-mouse-IgG antibody, are immobilized on different lines on the membrane.

[00129] Poly-SA was immobilized on the target or result line i.e. the first line in the direction of chromatography and should specifically capture particles bound to digoxigenylated and biotinylated peptide by means of the biotin binding. The anti-mouse IgG antibodies were immobilized on the control line i.e. the second line in the direction of chromatography. These anti-mouse IgG antibodies should capture all excess particles that were not bound on the result line (conjugates of anti-digoxigenin antibodies from the mouse and the silicate particles).

[00130] Depending on the test strip variant the application fleece was impregnated with the sample material to be examined i.e. with 1 µg/ml or 0 µg/ml of a biotinylated and digoxigenylated peptide. A 100 mM Hepes buffer pH 7.5 (50 mM NaCl, 70 mM urea, 1 mM EDTA, 2 % BPLA) was used to dilute the silicate particles and rewash the test strips. The silicate particles were diluted in Hepes buffer to a final concentration of 100 µg/ml.

[00131] Subsequently 60 µl of the described silicate particle dilution was pipetted on the reagent fleece and chromatographed for 10 min. Afterwards 40 µl Hepes buffer was pipetted onto the reagent fleece and chromatographed for a further 10 min. Finally the test strip was evaluated. Only the control line was visible in the absence of the peptide (= analyte) and in the presence of the peptide the result line was additionally visible.

[00132] The conjugates according to the invention of silicate particles colored with halogen-containing dyes and biomolecules (in this case anti-digoxigenin antibodies) are thus suitable as detection reagents in an immunological test strip.

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[00133] It is noted that terms like "preferably", "commonly", and "typically" are not utilized herein to limit the scope of the claimed invention or to imply that certain features are critical, essential, or even important to the structure or function of the claimed invention. Rather, these terms are merely intended to highlight alternative or additional features that may or may not be utilized in a particular embodiment of the present invention.

[00134] For the purposes of describing and defining the present invention it is noted that the term "substantially" is utilized herein to represent the inherent degree of uncertainty that may be attributed to any quantitative comparison, value, measurement, or other representation. The term "substantially" is also utilized herein to represent the degree by which a quantitative representation may vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

[00135] Having described the invention in detail and by reference to specific embodiments thereof, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims. More specifically, although some aspects of the present invention are identified herein as preferred or particularly advantageous, it is contemplated that the present invention is not necessarily limited to these preferred aspects of the invention.

[00136] What is claimed is:

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